PRIFYSGOL ABERYSTWYTH UNIVERSITY

Aberystwyth University

Linking microbial diversity and functionality of Arctic glacial surface habitats

Lutz, Stefanie ; Anesio, Alexandre M.; Edwards, Arwyn; Benning, Liane G.

Published in:
Environmental Microbiology

DOI:
10.1111/1462-2920.13494

Publication date:
2017

Citation for published version (APA):
Lutz, S., Anesio, A. M., Edwards, A., & Benning, L. G. (2017). Linking microbial diversity and functionality of Arctic glacial surface habitats. *Environmental Microbiology*, *19*(2), 551-565. https://doi.org/10.1111/1462-2920.13494

General rights
Copyright and moral rights for the publications made accessible in the Aberystwyth Research Portal (the Institutional Repository) are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the Aberystwyth Research Portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the Aberystwyth Research Portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

tel: +44 1970 62 2400
email: is@aber.ac.uk

Download date: 05. May. 2023

Accepted Article

Linking microbial diversity and functionality of Arctic glacial surface habitats

Stefanie Lutz[1,2*], Alexandre M. Anesio[3], Arwyn Edwards[4,5], Liane G. Benning[1,2]

[1]GFZ German Research Centre for Geosciences, Telegrafenberg, 14473 Potsdam, Germany

[2]Cohen Laboratories, School of Earth & Environment, University of Leeds, Leeds, LS2 9JT, UK

[3]Bristol Glaciology Centre, School of Geographical Sciences, University of Bristol, Bristol, BS8 1SS, UK

[4]Institute of Biological, Environmental & Rural Sciences (IBERS), Aberystwyth University, Aberystwyth, SY23 3FL, UK

[5]Interdisciplinary Centre for Environmental Microbiology, Aberystwyth University, SY23 3FL, UK

*corresponding author

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

This article is protected by copyright. All rights reserved.

Accepted Article

Originality-Significance Statement

Snow and ice algae are important primary colonisers and producers on glacial surfaces and they have a significant effect on the decrease of surface albedo. Here we have for the first time evaluated the role of these algae in association with the full microbial community composition in distinct glacial surface habitats and cross-correlated these data with analyses of important metabolites and physico-chemical parameters to identify drivers of community composition and function.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

Summary

Distinct microbial habitats on glacial surfaces are dominated by snow and ice algae, which are the critical players and the dominant primary colonisers and net producers during the melt season. Here we have for the first time evaluated the role of these algae in association with the full microbial community composition (*i.e.,* algae, bacteria, archaea) in distinct surface habitats and on twelve glaciers and permanent snow fields in Svalbard and Arctic Sweden. We cross-correlated these data with the analyses of specific metabolites such as fatty acids and pigments, and a full suite of potential critical physico-chemical parameters including major and minor nutrients, and trace metals. We show that correlations between single algal species, metabolites, and specific geochemical parameters can be used to unravel mixed metabolic signals in complex communities, further assign them to single species and infer their functionality. The data also clearly show that the production of metabolites in snow and ice algae is driven mainly by nitrogen and less so by phosphorus limitation. This is especially important for the synthesis of secondary carotenoids, which cause a darkening of glacial surfaces leading to a decrease in surface albedo and eventually higher melting rates.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

Introduction

The cryosphere covers about 10% of the Earth's surface and glaciers are an important component of the Earth's climate and freshwater system. Snow and ice habitats have recently been recognised as a biome dominated by microorganisms of all three domains of life, playing crucial roles in biogeochemical processes both on a local and global scale (Hodson *et al.*, 2008; Anesio and Laybourn-Parry, 2012; Lutz *et al.*, 2014, 2016). The presence of liquid water during the melt season makes glacial surfaces habitable and different microbial communities have been found to dominate 7 distinct surface habitats, which comprise clean snow, green snow, red snow, biofilms, clean ice, dirty ice, and cryoconite holes (Lutz *et al.*, 2014).

After the onset of melting, *clean snow* (white snow without visual presence of particles) undergoes a colour change due to snow algal blooming that turns the snow into *green snow* or *red snow*. The most abundant species in red snow belong to the *Chlamydomonaceae* with *Chlamydomonas* and *Chloromonas* as prominent genera (Leya, 2004). Dramatic morphological changes during their life cycles make an unambiguous species identification by microscopy very challenging. With molecular methods being established, it was shown that different strains had been misidentified as the universal *Chlamydomonas nivalis*, which therefore has to be treated as a collective taxon. The same applies to Chloromonas nivalis (Leya, 2013). Different aspects of snow algae ecology and physiology have been intensively studied in many polar and alpine settings including Svalbard (Müller *et al.*, 2001; Leya, 2004; Lutz, Anesio, Field, *et al.*, 2015; Lutz *et al.*, 2016), Iceland (Lutz, Anesio, Edwards, *et al.*, 2015), Alaska (Takeuchi, 2002), Greenland (Lutz *et al.*, 2014), the Himalayans

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

(Yoshimura *et al.*, 2006), the Rocky Mountains (Thomas and Duval, 1995), Antarctica (Fujii *et al.*, 2010; Remias *et al.*, 2013), and the European Alps (Remias *et al.*, 2005). However, only recently have we started to assess snow algal diversity through high-throughput molecular techniques in a few sites and or in specific habitats (Lutz, Anesio, Edwards, *et al.*, 2015; Lutz, Anesio, Field, *et al.*, 2015; Lutz *et al.*, 2016) but no study has evaluated yet the differences or similarities between these habitats and between different Arctic settings.

When the snow melts and the snow line moves further up the glacier and melting progresses, bare ice gets exposed. *Clean ice* (white ice without visual presence of particles) becomes colonised by filamentous algae that have only recently been described based on morphological and pigmentation traits (Remias *et al.*, 2009; Remias, Holzinger, *et al.*, 2012; Yallop *et al.*, 2012). The most described species are *Ancylonema nordenskiöldii* and *Mesotaenium berggrenii* that belong to the *Zygnematophyceae* (Remias *et al.*, 2009; Remias, Holzinger, *et al.*, 2012). They produce the brownish pigment purpurogallin carboxylic acid-6-O-b-D-glucopyranoside (Remias, Schwaiger, *et al.*, 2012), which together with accumulated mineral debris turn the ice surfaces into *dirty ice*. At the interface of snow and ice, *biofilms* can develop and these represent an ecotone between the snow and ice habitats.

Although snow and ice habitats dominate the largest proportion of glacial surfaces throughout the melt season, the microbial processes in *cryoconite holes* have been by far the more extensively studied habitats to date. This is because for years they have been considered the hotspots of biogeochemical processes on glacial surfaces (Stibal *et al.*, 2015; Cook *et al.*, 2016). Cryoconite holes are water-filled holes that

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

form by preferential melt of organic and inorganic dark particles and are dominated by cyanobacterial communities. Although not their preferential habitat, snow and ice algae may eventually end up in cryoconite holes due to melting processes and likely serve as an additional carbon and nutrient source for the heterotrophic communities, whereas cyanobacteria are the main primary producers (Anesio *et al.*, 2009).

Snow and ice algal blooms not only play a crucial role in carbon and nutrient cycling (Lutz *et al.*, 2014), but their pigmentation acts both as a protection from high irradiation (Remias *et al.*, 2005), but also darkens the surface of glaciers and ice sheets. This in turn leads to a decrease in surface albedo and subsequent further increased melt rates (Thomas and Duval, 1995; Yallop *et al.*, 2012; Takeuchi, 2013; Benning *et al.*, 2014; Lutz *et al.*, 2014, 2016; Musilova *et al.*, 2016). Yet, knowledge about the microbial community structures or functions in these different habitats is so far lacking.

To close this gap, we have evaluated the microbial community structure in the above described seven different habitats on twelve different glaciers and permanent snow fields in Svalbard and Arctic Sweden through high-throughput sequencing of the small subunit ribosomal RNA genes (16S and 18S), and combined these with analyses of specific metabolites that are crucial for a cryophilic life style such as fatty acids and pigments commonly found among the main primary producers of these habitats (Thompson Jr, 1996; Boussiba, 2000; Remias *et al.*, 2005). We cross-correlated these data with a full suite of potential critical physico-chemical parameters including major nutrients, trace metals and the prevalent mineralogy.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

In this study, we tested the hypotheses that (a) the microbial community composition and function within the different snow and ice habitats (Lutz *et al.*, 2014) are uniform across the Arctic and that (b) these habitats are affected by physico-chemical and geochemical parameters. Finally, based on our findings on contemporary surface microbial habitats we make predictions about processes that may affect past and future glacier surfaces.

Results

Community composition

We collected a total of 92 samples in the previously defined habitats (Lutz et al 2014), namely, clean snow, green snow, red snow, biofilms, clean ice, dirty ice and cryoconite holes comprising of 92 samples (Table S1). In all sequenced snow and ice samples (n=63) we found a low algal diversity with 9 species making up >99% of the algal communities (3 uncultured *Chlamydomonadaceae*, *Chloromonas polyptera*, *Chloromonas nivalis*, *Chloromonas alpina*, *Microglena sp*., *Raphidonema sempervirens* and *Ancylonema nordenskiöldii*) (Figure 2, Table S2 for full details, Table S13 for averages and p-values, $p<0.05$ = significant). Red snow samples (n=27) showed the lowest variation in species composition regardless of their geographic origin and all samples were dominated by an uncultured *Chlamydomonadaceae* species (labelled “2”). However, the species composition of green snow (n=14) varied between the two Arctic locations with higher relative abundance of *Microglena sp.* (20x) and *Raphidonema sempervirens* (10x) in

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an ‘Accepted Article’, doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

Svalbard and *Chloromonas polyptera* (10x) in Sweden. Biofilm samples (n=4) were characterized by higher relative abundances of *Chloromonas polyptera* compared to all other habitats in both Svalbard and Sweden. Dirty ice (n=10) and cryoconite holes (n=7) contained a higher relative abundance of filamentous ice algae with *Raphidonema sempervirens* and *Ancylonema nordenskiöldii* as the dominant taxa and a high similarity in community structure between the two habitats. Cryoconite holes also showed a higher ratio of fungi to algae than snow or ice samples (Table S3).

Bacteria (Figure 3, Table S4) were mainly represented by the phyla *Proteobacteria*, *Bacteriodetes* and *Cyanobacteria* in both geographic locations with samples more similar within one habitat than in-between habitats. *Bacteriodetes* showed higher relative abundance in snow and transition habitats with highest values for red snow and biofilms and lower abundances in dirty ice and cryoconite holes. *Proteobacteria* were abundant in all habitats, but *Alphaproteobacteria* were particularly more abundant in dirty ice, while *Betaproteobacteria* dominated in green snow. *Cyanobacteria* showed the highest relative abundance in dirty ice and cryoconite holes in Svalbard. Cryoconite holes were the habitats that showed the largest variation between locations with *Cyanobacteria* dominating in Svalbard and *Proteobacteria* in Sweden. Bacterial classes showed larger variations between all locations. Within the *Bacteriodetes*, *Sphingobacteria* were dominating in Sweden, and *Cytophagia*, *Saprospirae* and *Flavobacteria* in Svalbard. Within the *Cyanobacteria*, *Synechococcophycideae* were dominant in Svalbard (Table S4).

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

Archaea were detected in all analysed snow, ice and cryoconite samples (n=33). They showed very low diversity and the species composition was mainly made up by two groups, the *Nitrosophaerales* and *Methanobacteriales* (Table S5).

Aqueous and particulate geochemistry

We evaluated particulate total carbon, nitrogen, phosphorous and sulphur contents (TC, TN, TP, TS), particulate C/N/P ratios, as well as the full suite of dissolved major nutrients (DOC, PO_4, NO_3), dissolved minor and trace metal concentrations in the aqueous samples (e.g., K, Na, Ca, Fe, Mg, Mn), as well as the bulk mineralogy of each sample.

Most aqueous chemical data showed higher similarities within one location but not within a single habitat (see Table S7 for full details and Tables S13 and S14 for averages and statistical tests). DOC values were significantly and on average six times higher in Sweden compared to Svalbard, while all other nutrients showed no significant differences. In terms of dissolved elemental compositions, only Ca, Mg, Mn, Na and Cl were significantly higher in Svalbard compared to Sweden.

For C/N and C/P ratios in the particulates we found significant differences with samples clustering according to habitats within one location (Figure S4). For Svalbard and Sweden, C/N ratios were highest in red snow, followed by biofilms, similar values for dirty ice and cryoconite holes and lowest values in green snow (Table S8 and S13). Although the same trends could be observed in both locations, C/N ratios were generally higher in Sweden relative to the samples collected in Svalbard. All samples were above the optimal Redfield ratio for C/N of 6.6:1. We

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

This article is protected by copyright. All rights reserved.

Accepted Article

also found large differences for the C/P ratios. Most samples from Svalbard were below the optimal Redfield ratio of 106:1, while nearly all samples collected from Sweden were above this ratio.

$\delta^{15}N$ values were predominantly negative with no significant trends for habitats or geographic locations (Table S8). $\delta^{13}C$ values varied over a narrow range and significant trends could only be found for habitats in Svalbard with more negative values for red snow (-27.76‰ ± 1.52) and green snow (-28.21‰ ± 1.51‰) and higher for dirty ice (-26.28‰ ± 0.65) and cryoconite holes (-25.49‰ ± 0.25). There were no significant differences between the various habitats in Sweden and all samples showed a larger range in $\delta^{13}C$ values (between -30.03‰ and -21.80‰) compared to Svalbard.

The mineralogy slightly varied between the two locations. Quartz, plagioclase, mica and chlorite were present in all sites. In Svalbard we also found pyroxene and muscovite on all studied glaciers with calcite and dolomite on Austre Brøggerbreen and Feiringbreen, and hornblende and biotite in Arctic Sweden.

Metabolites

The relative abundance of functional groups corresponding to lipids, proteins and carbohydrates, clustered according to algal habitats (see Table S9 for full details and Table S13 for averages and statistical tests). Values for proteins and lipids were up to 5% higher in Sweden compared to Svalbard, but the same trends could be observed across the habitats within the respective locations. Concentrations of lipids

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

were significantly higher in red snow and biofilms, followed by dirty ice and green snow.

The most abundant fatty acids identified were the saturated fatty acids (SFA) C16:0 and C18:0, the monounsaturated fatty acids (MUFA) C16:1 and C18:1, and the polyunsaturated fatty acids (PUFA) C16:4, C18:2, C18:3 and C18:4 (Table S10 and S13). Principal component analysis (PCA) revealed clustering of the samples according to habitats and the fatty acids causing separation were C16:4, C18:3 and C18:4 for red snow, while C16:1, C18:1, and C15 branched for green snow, biofilms, dirty ice, and cryoconite holes (Figures 4 and S1). The relative abundance of PUFAs was significantly higher in red snow samples, followed by green snow, dirty ice, biofilms and cryoconite holes. MUFAs also showed significant differences between habitats and were highest in biofilms, followed by green snow, dirty ice, cryoconite holes and lowest in red snow. SFAs showed similar values for all samples and no significant differences between habitats. Dirty ice in Sweden showed 2x higher values of C18:3 and C18:4 compared to Svalbard. Cryoconite holes in Svalbard showed a 2x higher relative abundance of C15 branched fatty acids compared to dirty ice and all other habitats.

The relative abundance of secondary carotenoids (i.e., astaxanthin) varied significantly between habitats and they were highest in red snow, followed by biofilms, green snow, dirty ice and cryoconite holes (Table S11 and S13). Primary carotenoids also varied significantly between habitats and were highest in cryoconite holes, followed by dirty ice, green snow, and were lowest in red snow and biofilms. Chlorophyll concentrations were highest in dirty ice and green snow, followed by cryoconite holes, biofilms and lowest in red snow.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

Relationships between community composition, geochemistry and metabolites

We tested for relationships between microbial composition and geochemical parameters in the samples from the different glacial surface habitats. All correlations are summarised in Table 1. In brief, the two dominant uncultured *Chlamydomonadaceae* (1) and (2) (Figures 2 and S2, Table 1) and *Sphingobacteria* (Figure S3) were positively correlated with C/N ratios, and *Chloromonas polyptera, Chloromonas cf. alpina* and *Sphingobacteria* with DOC.

Canonical correspondence analysis (CCA) (Figure S2) and Pearson analyses (Table 1) showed a strong positive correlation for the relative abundance of the two uncultured *Chlamydomonadaceae* (1) and (2) with PUFAs, and between *Chloromonas cf. alpina* and MUFAs while a strong negative correlation was observed between *Raphidonema sempervirens* and PUFAs (Figure 4). Secondary carotenoids showed a strong positive correlation (Figure 5, Table 1) with the two uncultured *Chlamydomonadaceae* (1) and (2) and negative correlation with the uncultured *Chlamydomonadaceae* (3) and *Chloromonas cf. alpina*. *Raphidonema sempervirens* was also positively correlated with primary carotenoids (Figure 5). A positive correlation has been found for *Raphidonema sempervirens* and primary carotenoids and specifically for the pigments Antheraxanthin and Zeaxanthin with *Ancylonema nordenskiöldii*). The uncultured *Chlamydomonadaceae* (2) positively correlated with trans-Astaxanthin.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

The quantified metabolites correlated with particulate C/N/P ratios with a positive correlation for increasing C/N ratios and higher content of lipids, secondary carotenoids and PUFAs, and a positive correlation for increasing C/P and N/P ratios and higher lipid, protein and secondary carotenoid contents. The relative abundance of secondary carotenoids also positively correlated with the PUFAs C18:3 and C16:4.

Discussion

In this study, we have for the first time evaluated the microbial community composition in combination with a number of indicators of microbial functionality in several distinct glacial surface habitats and found clear differences between these habitats.

Algal community structure

The algal composition of red snow was very similar on all studied glaciers, as we have previously shown in a study where we specifically targeted red snow communities on glaciers in Greenland, Iceland, Svalbard and Sweden (Lutz *et al.*, 2016). In all these sites, the algal community in red snow was dominated by an uncultured *Chlamydomonadaceae* (2) regardless of variations in any metabolic or geochemical parameter differences between sites (Table S2).

The algal composition of green snow, as opposed to our findings in red snow, varied strongly between locations with higher relative abundance of *Raphidonema*

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

sempervirens and *Microglena sp.* in Svalbard, and *Chloromonas polyptera* in Sweden. It is important to note that the area covered of green snow on glacial surfaces was by far smaller compared to the red snow distribution, which was the dominant snow algal habitat on all glaciers surveyed in this study. In green snow the large variations in community composition may imply that the green snow community is more sensitive to its physico-chemical environment. *Chloromonas polyptera* and *Chloromonas cf. alpina* seem to thrive in habitats with high water activity, namely green snow and biofilms, which also seem to accumulate a higher mineral content. *Chloromonas polyptera* was also found to be highly abundant in glacial snow in Iceland after heavy rain (Lutz, Anesio, Edwards, *et al.*, 2015) and hence wet snow conditions. Both species were positively correlated with DOC (Figure S2 and Table 1), which suggests that they either prefer environments with more organic carbon or are effective producers of DOC. However, the variations in community composition of green snow may also be explained by potential different sources for the colonising species. In Svalbard a high concentration of elements likely derived from sea spray input (e.g., SO_4, Cl) or through the weathering of the bedrocks (e.g., Na, Ca, Mg; Table S7) may indicate a different source for the development of green snow communities.

Surprisingly, the dirty ice habitat did not reveal a high abundance of the previously described ice algae *Ancylonema nordenskiöldii* and *Mesotaenium berggrenii* (Table S2) (Remias *et al.*, 2009; Remias, Holzinger, *et al.*, 2012; Yallop *et al.*, 2012). Only a few samples from Svalbard showed a higher relative abundance of *Ancylonema nordenskiöldii* (up to 65%) and only very few sequences of *Mesotaenium berggrenii* (<0.01%). In our dirty ice samples the dominant algal species (between 10 and 88%)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

was *Raphidonema sempervirens,* better known as a permafrost algae (Stibal and Elster, 2005), and an uncultured *Chlamydomonadaceae* (2). The latter was most likely transferred to the ice surface from the melting snowpack above the ice as this was also the dominant species in the snow. Most algal species identification in snow and ice environments have so far been done by microscopy and only very limited sequencing data are available (Leya, 2004). Therefore there is a potential for misassignments between microscopy and sequencing information. Another reason for the apparent discrepancy between the dominant ice algae in our study and previous investigations could be a potential dominance of external input (i.e., the uncultured *Chlamydomonadaceae* (2) from the melting snowpack, and *Raphidonema sempervirens* from the nearby soil) over *in-situ* thriving of *Ancylonema nordenskiöldii* and *Mesotaenium berggrenii* on dirty ice at the time of sampling.

Bacterial community structure

Overall far more sequencing data on bacteria from various glacial habitats are available in the literature compared to algae (Larose *et al.*, 2010; Edwards *et al.*, 2011; Hell *et al.*, 2013; Cameron *et al.*, 2014; Musilova *et al.*, 2015). However, the data are primarily focussed on bacterial communities in clean snow or cryoconite holes. The transitions between habitats in our samples are also reflected in the changing bacterial community. For example, *Bacteriodetes* were dominant in red snow and biofilms in all locations (Figure 3 and Table S4). They are known to play pivotal roles in the degradation of organic matter (Thomas *et al.*, 2011). The high relative abundance of *Bacteriodetes* in the red snow samples as well as their positive correlation with DOC may underpin the importance of red snow algal blooms as

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

This article is protected by copyright. All rights reserved.

Accepted Article

carbon source on glacial surfaces. We also found a decline in the relative abundance of *Bacteriodetes* when transitioning from snow to ice.

Within the *Bacteriodetes*, *Sphingobacteria* were only highly abundant in Sweden. They are known to be capable of degrading complex organic compounds (Thomas *et al.*, 2011) and were positively correlated with TC and DOC (Figure S3 and Table 1). This could be explained by the vicinity to higher plants and the high abundance of pollen in our samples (Table S8). In contrast, in Svalbard there was a higher relative abundance of species with pathogenic life styles including *Cytophagia, Flavobacteria* and *Saprospirae*, which are known to be microalgal pathogens (Salomon and Imai, 2006). In addition, the fungi *Chytridiomycota* also showed a higher abundance in Svalbard which have been found to be involved in parasitic associations with microalgae (Ibelings *et al.*, 2004). This may imply the presence of important nutrient recycling processes based on non-mutual symbiotic relationships between algae and other members of the microbial community in Svalbard.

Dirty ice was more dominated by *Proteobacteria* and we found varying compositions for cryoconite holes, which can be considered as the “end members” of supraglacial habitats. Cameron *et al.* (2014) also found a higher relative abundance of *Proteobacteria* (60%) in their summer snow samples from Greenland and other studies have reported a strong representation of the supraglacial communities including snow, ice and cryoconite holes by *Proteobacteria* (Harding *et al.*, 2011; Hell *et al.*, 2013; Edwards *et al.*, 2014).

Green snow and dirty ice habitats were also dominated by *Proteobacteria*. Among these, *Betaproteobacteria* were dominant in green snow, whereas *Alphaproteobacteria* were dominant in dirty ice. Both these habitats contain on

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an ‘Accepted Article’, doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

average two times lower amounts of organic matter (based on particulate organic carbon and DOC data) compared to red snow, but with different selection pressure on the bacterial class level. Among others, this could be associated with the differences in the physical state of the water (snow *vs.* ice) or the main primary producers. *Alphaproteobacteria* have been shown to grow at low carbon and nutrient concentrations (Eiler *et al.*, 2003), which are more typical for ice surfaces. On the other hand, *Betaproteobacteria* often use anaerobic decomposition products of organic-matter. This may be more common in green snow, which mostly forms close to glacial snouts and at rock interfaces, and therefore is potentially more influenced by an excess of organic matter and decomposition processes driving O_2 consumption. Green snow is also usually much 'wetter' and more debris rich compared to red snow, and thus appears to likely have experienced more melting.

The dominating phyla in cryoconite holes were *Cyanobacteria* in Svalbard and *Proteobacteria* in Sweden. There is a large similarity between the community composition of dirty ice and cryoconite holes within one location, which also reflects the transition between those habitats. Cyanobacteria were only abundant in dirty ice and cryoconite holes in Svalbard, but not in Sweden. Within the *Synechococcophycidae*, the *Leptolyngbya* were dominant, which corroborates the findings of Kaštovská *et al.* (2005).

Archaeal community structure

Archaea were detected in all analysed samples and showed very low diversity (Table S5). The species composition was mainly represented by two taxa, the

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

This article is protected by copyright. All rights reserved.

Accepted Article

Nitrosophaerales and *Methanobacteriales*. *Nitrosphaerales* are important ammonia-oxidizers (Tourna *et al.*, 2011; Zarsky *et al.*, 2013; Stieglmeier *et al.*, 2014), whereas *Methanobacteriales* are anaerobic methanogens. We could not establish any trends for their distribution within the studied habitats, but their seemingly cosmopolitan distribution in supraglacial environments may indicate an important role in the nitrogen cycle of these habitats.

Community function

Most of our samples showed C/N ratios (Figures 6 and S4, Tables 1 and S8) above the Redfield ratio (C/N/P:106/16/1) which has been described to be optimal for algal exponential growth and incorporation of macronutrients into reproductive biomolecules including phosphate-rich ribosomes (Sterner and Elser, 2002; Klausmeier *et al.*, 2004). If nitrogen or phosphorus become limiting, carbon incorporation is preferentially stored as starch or lipids leading to a high C/N cellular ratio (James *et al.*, 2011). This matches previous finding confirming nitrogen limitation by snow algae (Jones and Deblois, 1987; Jones and Tranter, 1989). This is further reflected in the low N/P ratios found across our samples (Figure S4, Tables 1 and S8). Low N/P ratios have also been observed in freshwater and marine phytoplankton during blooms since fast-growing cells require phosphorus for ribosomes (Sterner and Elser, 2002; Klausmeier *et al.*, 2004). In contrast, most of our samples showed low C/P ratios and hence less P limitation. Only red snow samples from Sweden showed high C/P ratios and therefore indicating co-limitation of nitrogen and phosphorus in this particular habitat. However, it cannot be excluded that the high abundance of pollen in Sweden contributed to a higher TC and

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

This article is protected by copyright. All rights reserved.

Accepted Article

therefore higher C/N and C/P ratios and also a higher relative abundance of proteins compared to Svalbard (Figure 6).

Lipids were highest in red snow in both Svalbard and Sweden and were positively correlated with C/N ratios (Figure 6, Tables 1). Accumulation of lipids has been found to be a consequence of nitrogen limitation in cryophilic algae (Pichrtová *et al.*, 2014) since metabolism is shifted to the production of nitrogen-free metabolites during nitrogen limiting conditions. Triacylglycerol (TGA) neutral lipids are often synthesized in response to high light or nutrient deprivation (James *et al.*, 2011). Lipid bodies can also store dissolved astaxanthin, which may be a valuable long-term energy source. In addition, C/N ratios in red snow could have also been increased as an effect of the cyst formation of mature snow algal cells and incorporation of nitrogen- and phosphorus-free carotenoids.

The abundance of C, N, and P also positively correlated with the production of important cryophilic biomolecules including fatty acids and pigments (Figure 6 and Table 1). Algae can change their fatty acid composition in response to changing environmental conditions including temperature, pH and nutrients (James *et al.*, 2011). PUFAs (in particular C16:4 and C18:3) were dominant in red snow in both Sweden and Svalbard and strongly correlated with the presence of the two dominant uncultured *Chlamydomonadaceae* (1) and (2) and high C/N ratios (Figures 4, Table 1). This is in accordance with laboratory investigations on nitrogen deprived *Chlamydomonas reinhardtii* starch mutants, which produced more MUFAs and also PUFAs including C18:3 compared to nitrogen-sufficient conditions. MUFAs in phospholipids have been found to maintain membrane fluidity (Bidigare *et al.*, 1993; Leya, 2004). These compounds were most abundant in green snow and biofilm

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

samples, the two habitats with the highest water activity and therefore more sensitive to freezing. C15 branched fatty acids are known as biomarkers for bacteria (Zelles, 1999) and high abundance in dirty ice and in particular in cryoconite holes is likely due to cyanobacterial presence.

Chlorophyll concentrations were higher in dirty ice and green snow compared to red snow. The carotenoid-rich algae are still photosynthetically active (Lutz *et al.*, 2014), however, they are likely less active than the chlorophyll-rich algae. In a previous publication we have shown that the metabolism within the red snow habitat is more directed towards storage and reserve metabolites, whereas the metabolic profile within the green snow habitat was characterized by metabolites involved in growth and proliferation (Lutz, Anesio, Field, *et al.*, 2015).

Secondary carotenoid production was positively correlated with high C/N, C/P and N/P ratios (Figure 6, Table 1). Our data show that snow and ice algae use different carotenoid synthesis strategies. The main snow algal species showed a strong correlation with secondary carotenoids, whereas there was a negative correlation between secondary carotenoids for the ice algae *Raphidonema sempervirens* yet a positive correlation for primary carotenoids (Figure 5 and Table 1). The second most abundant ice algae *Ancylonema nordenskiöldii* was also positively correlated with the two primary carotenoids Zeaxanthin and Antheraxanthin, which may imply that these species are using the xanthophyll cycle instead. This cycle involves the enzymatic removal of epoxy groups from Violaxanthin and Antheraxanthin, which results in the de-epoxidised xantophyll Zeaxanthin (Remias, 2012). The production of secondary carotenoids has not been shown yet for ice algae (Leya *et al.*, 2009) and the negative correlation between *Raphidonema sempervirens* and secondary

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

carotenoids as well as PUFAs suggests the absence of a secondary carotenoid production pathway in ice algae.

Nitrogen isotopes did not show any differences between habitats or locations and were predominantly negative suggesting an atmospheric nitrogen source for all samples (Figure S5, Table S8). In contrast, carbon isotopes were lower in snow than ice habitats in Svalbard. This could indicate different photosynthetic mechanism and/or more input of allochthonous carbon in the snow samples since the $\delta^{13}C$ values reflect those of vascular plants (Farquhar *et al.*, 1989). No significant variations could be found for Sweden, likely due to the presence of pollen as a confounding factor.

Combining all these data reveals that the production of a range of quantified metabolites in snow and ice algae in both Svalbard and Sweden was driven mainly by the availability of N and less by P but likely also changes the intracellular C/N/P ratios. Correlations between metabolites, geochemistry and single species could be used to unravel mixed metabolic signals in complex communities. Furthermore, this could be used to assign them to single species and make assumptions about their role in the respective habitat. However, nutrients stoichiometry may not be the only factor affecting community structure, and other parameters including the physical state of the water will likely also play an important role.

Implications for the past and future of glacial surfaces

The environment determines the distribution and structure of algal communities, but in return algal blooms also alter their environment after successful colonisation of the

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

This article is protected by copyright. All rights reserved.

Accepted Article

different surface glacial habitats. Algal communities exhibit short generation times and are able to adapt their metabolic inventory quickly as response to environmental conditions that change over relatively short time and space. Studying their ecological patterns and how these may be altered can give us a valuable outlook into how Arctic glacial and ice sheet surface ecosystems may respond to a changing climate. Climate-warming is predicted to have a large impact on glacial runoff and this will, in turn, also have an effect on organic carbon fluxes from glacial surface to downstream ecosystems (Hood *et al.*, 2015), particularly as the contribution of DOC from all mountain glaciers is higher (56% of total DOC export) than from the Greenland and Antarctic ice sheets (Hood *et al.*, 2015). With regard to carbon and nutrient export, snow microbial communities are the prime habitat feeding other supraglacial, subglacial and periglacial habitats (Wynn *et al.*, 2007; Hodson *et al.*, 2008; Schütte *et al.*, 2009; Telling *et al.*, 2011), yet despite the plethora of data discussed above and stemming from various samples from 92 different samples on 12 Arctic glaciers, there are still many open questions about their functions.

We did however show that the production of the quantified metabolites is driven by availability of major nutrients, which is especially important for the production of secondary carotenoids, which in turn can cause a darkening of the glacial surfaces and therefore a decrease in surface albedo (Thomas and Duval, 1995; Takeuchi, 2013; Benning *et al.*, 2014). We show that secondary carotenoid production is positively correlated with nitrogen limitation. Considering lower pre-anthropogenic nitrogen levels, this effect may have been even stronger during past inter-glaciation events and it may have contributed to local heat retention due to lower surface albedos. In addition, this could also provide the other glacial microbial communities

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

This article is protected by copyright. All rights reserved.

Accepted Article

with water in its liquid state, which is crucial for life in general. Possibly, this effect also extends the active growth season of snow algae due to earlier and prolonged availability of liquid water. Finally, increased nitrogen deposition in the future due to continual and increased anthropogenic input may lead to higher cell numbers eventually reaching limiting conditions but in that case accelerating the glacial and ice sheet darkening effects even more.

Experimental procedures

A total of 92 samples (10 clean snow, 34 red snow, 15 green snow, 4 biofilm, 7 clean ice, 15 dirty ice, and 7 cryoconite hole sediment samples) were collected across 6 glaciers in Svalbard and 6 glaciers and snow fields in the Arctic Sweden (Tarfala Valley, Figure 1, Tables S1, S13, S14). The glaciers in Svalbard (Vestre Brøggerbreen, Midtre Lovénbreen, Austre Brøggerbreen, Pedersenbreen, Austre Lovénbreen and Feiringbreen) were sampled in July and August 2013, and Storglaciären, Rabot, Liljetopsrännan, SE-Kasskasatjåkkå, Björling and nearby permanent snow fields in the Arctic Sweden were sampled in July 2013 and July 2014.

Details of the sampling methods, the field measurements and the majority of the analyses have been described previously (Lutz *et al.*, 2014, 2016; Lutz, Anesio, Edwards, *et al.*, 2015) and can be found in the supplementary information. In brief, at each sampling site we measured pH, conductivity and temperature with a daily-calibrated meter (Hanna instruments, HI 98129). PAR, UV irradiation and surface albedo (400-700 nm range) were measured using a radiometer (SolarLight,

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13494

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

PMA2100). Next to the field measurement sites, samples were collected either in sterile centrifuge tubes or sterile *Whirl-Pak*® bags (for genomic and inorganic analyses) or in pre-ashed glass jars (450◦C >4 h) for organic analyses. Samples were slowly melted at room temperature and processed for further analyses (*i.e.*, filtered, concentrated, acidified) within ~ 8 hours after collection. Samples for DNA and particulate organic analyses (i.e., pigments, fatty acids) were flash-frozen in liquid nitrogen and stored at -80°C until analysed. All inorganic samples were stored cold (4°C) and in the dark. For imaging by light microscopy unconcentrated samples were preserved in 2.5% glutaraldehyde and images recorded through 63x and 100x objectives.

Aqueous compounds were analysed by Ion Chromatography (IC, Dionex; anions), by inductively coupled plasma mass spectrometry (ICP-MS, Agilent; cations), while dissolved organic carbon (DOC) was analysed on a total organic carbon analyser (TOC, Shimadzu) and dissolved phosphate by segmented flow-injection analyses (AutoAnalyser3, Seal Analytical).

Samples that contained enough solid particulates were analysed for total carbon (TC), total nitrogen (TN), total sulphur (TS), as well as $\delta^{15}N$ and $\delta^{13}C$, by pyrolysis at 1500 °C (Vario Pyro Cube, Elementar Inc.) followed by mass spectrometry (Isoprime Mass Spectrometer). Particulate phosphorus was extracted by ashing of the samples at 550°C for 2 h and incubating in 1 M HCl for 16 h according to extraction step V in Ruttenberg *et al.* (2009) and quantified by segmented flow-injection analyses (AutoAnalyser3, Seal Analytical).

The relative abundance of functional groups corresponding to proteins, lipids and carbohydrates was evaluated by Fourier transform infrared spectroscopy (FTIR, A2

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

Technology Microlab). Pigments were extracted in dimethylformamide and analysed using high-pressure liquid chromatography (HPLC, Agilent 1220 Infinity). Fatty acids were extracted in dichloromethanol:methanol (2:1, v:v), transesterified in methanolic HCl, followed by extraction in isohexane and analysed by gas chromatography mass spectrometry (GC-MS, Thermo Scientific, Trace1300, ISQ Single Quadrupole).

Of the seven habitats studied, enough particulates for DNA extraction was only available in the green snow, red snow, biofilm, dirty ice and cryoconite hole samples. Total DNA was extracted using the PowerSoil® DNA isolation kit (MoBio Laboratories) and three amplicon libraries were prepared for bacteria, eukaryotes, and archaea. Libraries were sequenced on the Ion Torrent Personal Genome Machine. Raw sequences were processed and OTU tables were constructed in QIIME (Caporaso *et al.*, 2010). Principal component analysis (PCA), canonical correspondence analysis (CCA) and Pearson correlations were carried out in PAST v3.06 (Hammer *et al.*, 2012) and one-way analysis of variance (ANOVA) was done in SPSS v19 (IBM). One-way ANOVA was chosen over two-way ANOVA due to the uneven sampling number for the habitats and locations and to avoid skewness of the statistical analyses. Samples were compared either across habitats within one location or across locations within one habitat by one-way ANOVA. Sequences were deposited in the ENA under accession number PRJEB14001.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

Acknowledgments

The authors would like to thank Andrew Detheridge (Aberystwyth University), and Christy Waterfall and Jane Coghill (University of Bristol) for help with the DNA sequencing. We would like to acknowledge Anthony Stockdale (University of Leeds) for the phosphorus analysis, Fiona Gill for help with the fatty acids analysis and Rob J. Newton for help with the particulate carbon and nitrogen analyses. This work was funded by a University of Leeds grant to SL and LGB, by a Young Explorers grant from National Geographic (GEFNEY73-13) and a President's Fund for Research Visit grant from the Society for General Microbiology granted (PF13/16) to SL, the UK Natural Environment Research Council grants NE/J022365/1 to LGB and NE/J02399X/1 to AMA, and by a grant from the European Union Seventh Framework Program INTERACT (grant no 262693) to LGB. We would also like to thank the scientific staff at the Tarfala and NERC Arctic research stations for great field support.

Conflict of interest

The authors declare that they have no competing financial interests.

References

Anesio, A.M., Hodson, A.J., Fritz, A., Psenner, R., and Sattler, B. (2009) High microbial activity on glaciers: importance to the global carbon cycle. *Glob. Chang. Biol.* **15**: 955–960.

Anesio, A.M. and Laybourn-Parry, J. (2012) Glaciers and ice sheets as a biome. *Trends Ecol. Evol.* **27**: 219–225.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

Benning, L.G., Anesio, A.M., Lutz, S., and Tranter, M. (2014) Biological impact on Greenland's albedo. *Nat. Geosci* **7**: 691.

Bidigare, R.R., Ondrusek, M.E., Kennicutt, M.C., Iturriaga, R., Harvey, H.R., Hoham, R.W., and Macko, S.A. (1993) Evidence for a photoprotective function for secondary carotenoids of snow algae. *J. Phycol.* **29**: 427–434.

Boussiba, S. (2000) Carotenogenesis in the green alga Haematococcus pluvialis: cellular physiology and stress response. *Physiol. Plant.* **108**: 111–117.

Cameron, K.A., Hagedorn, B., Dieser, M., Christner, B.C., Choquette, K., Sletten, R., et al. (2014) Diversity and potential sources of microbiota associated with snow on western portions of the Greenland Ice Sheet. *Environ. Microbiol.*

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **7**: 335–336.

Cook, J., Edwards, A., Takeuchi, N., and Irvine-Fynn, T. (2016) Cryoconite: The dark biological secret of the cryosphere. *Prog. Phys. Geogr.* **40**: 66–111.

Edwards, A., Anesio, A.M., Rassner, S.M., Sattler, B., Hubbard, B., Perkins, W.T., et al. (2011) Possible interactions between bacterial diversity, microbial activity and supraglacial hydrology of cryoconite holes in Svalbard. *ISME J.* **5**: 150–160.

Edwards, A., Mur, L.A.J., Girdwood, S.E., Anesio, A.M., Stibal, M., Rassner, S.M.E., et al. (2014) Coupled cryoconite ecosystem structure–function relationships are revealed by comparing bacterial communities in alpine and Arctic glaciers. *FEMS Microbiol. Ecol.* **89**: 222–237.

Eiler, A., Langenheder, S., Bertilsson, S., and Tranvik, L.J. (2003) Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. *Appl. Environ. Microbiol.* **69**: 3701–3709.

Farquhar, G.D., Ehleringer, J.R., and Hubick, K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Biol.* **40**: 503–537.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

This article is protected by copyright. All rights reserved.

Fujii, M., Takano, Y., Kojima, H., Hoshino, T., Tanaka, R., and Fukui, M. (2010) Microbial community structure, pigment composition, and nitrogen source of red snow in Antarctica. *Microb. Ecol.* **59**: 466–475.

Hammer, O., Harper, D.A.T., and Ryan, P.D. (2012) PAST: paleontological statistics software package for education and data analysis. *Paleontol Electron* **4.**:

Harding, T., Jungblut, A.D., Lovejoy, C., and Vincent, W.F. (2011) Microbes in high Arctic snow and implications for the cold biosphere. *Appl. Environ. Microbiol.* **77**: 3234–3243.

Hell, K., Edwards, A., Zarsky, J., Podmirseg, S.M., Girdwood, S., Pachebat, J.A., et al. (2013) The dynamic bacterial communities of a melting High Arctic glacier snowpack. *ISME J.* **7**: 1814–1826.

Hodson, A., Anesio, A.M., Tranter, M., Fountain, A., Osborn, M., Priscu, J., et al. (2008) Glacial ecosystems. *Ecol. Monogr.* **78**: 41–67.

Hood, E., Battin, T.J., Fellman, J., O'Neel, S., and Spencer, R.G.M. (2015) Storage and release of organic carbon from glaciers and ice sheets. *Nat. Geosci.* **8**: 91–96.

Ibelings, B.W., De Bruin, A., Kagami, M., Rijkeboer, M., Brehm, M., and Donk, E. Van (2004) Host parasite interactions between freshwater phytoplankton and chytrid fungi (Chytridiomycota). *J. Phycol.* **40**: 437–453.

James, G.O., Hocart, C.H., Hillier, W., Chen, H., Kordbacheh, F., Price, G.D., and Djordjevic, M.A. (2011) Fatty acid profiling of Chlamydomonas reinhardtii under nitrogen deprivation. *Bioresour. Technol.* **102**: 3343–3351.

Jones, H.G. and Deblois, C. (1987) Chemical dynamics of N-containing ionic species in a boreal forest snowcover during the spring melt period. *Hydrol. Process.* **1**: 271–282.

Jones, H.G. and Tranter, M. (1989) Interactions Between Meltwater and Organic-Rich Particulate Material in Boreal Forest Snowpacks: Evidence for Both Physico-Chemical and Microbiological Influences.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Kaštovská, K., Elster, J., Stibal, M., and Šantrůčková, H. (2005) Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (high Arctic). *Microb. Ecol.* **50**: 396–407.

Klausmeier, C.A., Litchman, E., Daufresne, T., and Levin, S.A. (2004) Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature* **429**: 171–174.

Larose, C., Berger, S., Ferrari, C., Navarro, E., Dommergue, A., Schneider, D., and Vogel, T.M. (2010) Microbial sequences retrieved from environmental samples from seasonal Arctic snow and meltwater from Svalbard, Norway. *Extremophiles* **14**: 205–212.

Leya, T. (2004) Feldstudien und genetische Untersuchungen zur Kryophilie der Schneealgen Nordwestspitzbergens Shaker Aachen.

Leya, T. (2013) Snow algae: adaptation strategies to survive on snow and ice. In, *Polyextremophiles*. Springer, pp. 401–423.

Leya, T., Rahn, A., Lütz, C., and Remias, D. (2009) Response of arctic snow and permafrost algae to high light and nitrogen stress by changes in pigment composition and applied aspects for biotechnology. *FEMS Microbiol. Ecol.* **67**: 432–443.

Lutz, S., Anesio, A.M., Edwards, A., and Benning, L.G. (2015) Microbial diversity on icelandic glaciers and ice caps. *Front. Microbiol.* **6.**:

Lutz, S., Anesio, A.M., Field, K., and Benning, L.G. (2015) Integrated “Omics”, targeted metabolite and single-cell analyses of arctic snow algae functionality and adaptability. *Front. Microbiol.* **6.**:

Lutz, S., Anesio, A.M., Jorge Villar, S.E., and Benning, L.G. (2014) Variations of algal communities cause darkening of a Greenland glacier. *FEMS Microbiol. Ecol.* **89**: 402–414.

Lutz, S., Anesio, A.M., Raiswell, R., Edwards, A., Newton, R.J., Gill, F., and Benning, L.G. (2016) The biogeography of red snow microbiomes and their role in melting arctic glaciers. *Nat. Commun.* **7.**:

Müller, T., Leya, T., and Fuhr, G. (2001) Persistent snow algal fields in Spitsbergen: field observations and a hypothesis about the annual cell circulation. *Arctic, Antarct. Alp. Res.* 42–51.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an ‘Accepted Article’, doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Musilova, M., Tranter, M., Bamber, J.L., Takeuchi, N., and Anesio, A.M. (2016) Experimental evidence that microbial activity lowers the albedo of glaciers. *Lett. Geochemical Perspect. Lett. Geochem. Persp. Let. Geochem. Persp. Let* **2.**:

Musilova, M., Tranter, M., Bennett, S.A., Wadham, J., and Anesio, A.M. (2015) Stable microbial community composition on the Greenland Ice Sheet. *Front. Microbiol.* **6.**:

Pichrtová, M., Kulichová, J., and Holzinger, A. (2014) Nitrogen Limitation and Slow Drying Induce Desiccation Tolerance in Conjugating Green Algae (Zygnematophyceae, Streptophyta) from Polar Habitats. *PLoS One* **9**: e113137.

Remias, D. (2012) Cell structure and physiology of alpine snow and ice algae Springer, Vienna.

Remias, D., Holzinger, A., Aigner, S., and Lütz, C. (2012) Ecophysiology and ultrastructure of Ancylonema nordenskiöldii (Zygnematales, Streptophyta), causing brown ice on glaciers in Svalbard (high arctic). *Polar Biol.* **35**: 1–10.

Remias, D., Holzinger, A., and Lütz, C. (2009) Physiology, ultrastructure and habitat of the ice alga Mesotaenium berggrenii (Zygnemaphyceae, Chlorophyta) from glaciers in the European Alps. *J. Inf.* **48.**:

Remias, D., Lütz-Meindl, U., and Lütz, C. (2005) Photosynthesis, pigments and ultrastructure of the alpine snow alga Chlamydomonas nivalis. *Eur. J. Phycol.* **40**: 259–268.

Remias, D., Schwaiger, S., Aigner, S., Leya, T., Stuppner, H., and Lütz, C. (2012) Characterization of an UV- and VIS-absorbing, purpurogallin-derived secondary pigment new to algae and highly abundant in Mesotaenium berggrenii (Zygnematophyceae, Chlorophyta), an extremophyte living on glaciers. *FEMS Microbiol. Ecol.* **79**: 638–648.

Remias, D., Wastian, H., Lütz, C., and Leya, T. (2013) Insights into the biology and phylogeny of Chloromonas polyptera (Chlorophyta), an alga causing orange snow in Maritime Antarctica. *Antarct. Sci.* 1–9.

Ruttenberg, K.C., Ogawa, N.O., Tamburini, F., Briggs, R.A., Colasacco, N.D., and Joyce, E. (2009) Improved, high-throughput approach for phosphorus speciation in natural sediments via the

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

SEDEX sequential extraction method. *Limnol. Oceanogr. Methods* **7**: 319–333.

Salomon, P.S. and Imai, I. (2006) Pathogens of harmful microalgae. In, *Ecology of Harmful Algae*. Springer, pp. 271–282.

Schütte, U.M.E., Abdo, Z., Bent, S.J., Williams, C.J., Schneider, G.M., Solheim, B., and Forney, L.J. (2009) Bacterial succession in a glacier foreland of the High Arctic. *ISME J.* **3**: 1258–1268.

Sterner, R.W. and Elser, J.J. (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere Princeton University Press.

Stibal, M. and Elster, J. (2005) Growth and morphology variation as a response to changing environmental factors in two Arctic species of Raphidonema (Trebouxiophyceae) from snow and soil. *Polar Biol.* **28**: 558–567.

Stibal, M., Schostag, M., Cameron, K.A., Hansen, L.H., Chandler, D.M., Wadham, J.L., and Jacobsen, C.S. (2015) Different bulk and active bacterial communities in cryoconite from the margin and interior of the Greenland ice sheet. *Environ. Microbiol. Rep.* **7**: 293–300.

Stieglmeier, M., Klingl, A., Alves, R.J.E., Simon, K.-M.R., Melcher, M., Leisch, N., and Schleper, C. (2014) Nitrososphaera viennensis sp. nov., an aerobic and mesophilic ammonia-oxidizing archaeon from soil and member of the archaeal phylum Thaumarchaeota. *Int. J. Syst. Evol. Microbiol.* **64**: 2738–2752.

Takeuchi, N. (2013) Seasonal and altitudinal variations in snow algal communities on an Alaskan glacier (Gulkana glacier in the Alaska range). *Environ. Res. Lett.* **8**: 35002.

Takeuchi, N. (2002) The altitudinal distribution of snow algae on an Alaska glacier (Gulkana Glacier in the Alaska Range). *Hydrol. Process.* **15**: 3447–3459.

Telling, J., Anesio, A.M., Tranter, M., Irvine-Fynn, T., Hodson, A., Butler, C., and Wadham, J. (2011) Nitrogen fixation on Arctic glaciers, Svalbard. *J. Geophys. Res.* **116**: G03039.

Thomas, F., Hehemann, J.-H., Rebuffet, E., Czjzek, M., and Michel, G. (2011) Environmental and gut bacteroidetes: the food connection. *Front. Microbiol.* **2.**:

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Thomas, W.H. and Duval, B. (1995) Sierra Nevada, California, USA, snow algae: snow albedo changes, algal-bacterial interrelationships, and ultraviolet radiation effects. *Arct. Alp. Res.* **27**: 389–399.

Thompson Jr, G.A. (1996) Lipids and membrane function in green algae. *Biochim. Biophys. Acta (BBA)-Lipids Lipid Metab.* **1302**: 17–45.

Tourna, M., Stieglmeier, M., Spang, A., Könneke, M., Schintlmeister, A., Urich, T., et al. (2011) Nitrososphaera viennensis, an ammonia oxidizing archaeon from soil. *Proc. Natl. Acad. Sci.* **108**: 8420–8425.

Wynn, P.M., Hodson, A.J., Heaton, T.H.E., and Chenery, S.R. (2007) Nitrate production beneath a High Arctic glacier, Svalbard. *Chem. Geol.* **244**: 88–102.

Yallop, M.L., Anesio, A.M., Perkins, R.G., Cook, J., Telling, J., Fagan, D., et al. (2012) Photophysiology and albedo-changing potential of the ice algal community on the surface of the Greenland ice sheet. *ISME J.* **6**: 2302–2313.

Yoshimura, Y., Kohshima, S., Takeuchi, N., Seko, K., and Fujita, K. (2006) Snow algae in a Himalayan ice core: new environmental markers for ice-core analyses and their correlation with summer mass balance. *Ann. Glaciol.* **43**: 148–153.

Zarsky, J.D., Stibal, M., Hodson, A., Sattler, B., Schostag, M., Hansen, L.H., et al. (2013) Large cryoconite aggregates on a Svalbard glacier support a diverse microbial community including ammonia-oxidizing archaea. *Environ. Res. Lett.* **8**: 35044.

Zelles, L. (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biol. Fertil. Soils* **29**: 111–129.

Accepted Article

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

Tables

Table 1: Statistical analysis of correlations between all analysed biological and geochemical compounds in all samples. Shown are Pearson correlation factors (r) and significance of correlations (p). Correlations with $p < 0.05$ were considered to be significant. DOC=dissolved organic carbon; K = potassium, Fe = iron, Mn = manganese; C = total particulate organic carbon, N = total particulate nitrogen; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

Figures

Figure 1: Map showing the 2 geographic locations and 12 glaciers and snow fields sampled in Svalbard and Arctic Sweden. At each marked site various habitats were sampled (full details see Table S1).

Figure 2: Principal component analysis of algal species in Svalbard and Arctic Sweden samples revealing taxonomic distance between samples and showing the main taxa causing the separations. Samples cluster according to habitats in all locations. Bar charts show average species composition within one habitat and location with the numbers in brackets at the bottom of each chart representing sample numbers per habitat.

Figure 3: Principal component analysis of bacterial phyla in Svalbard and Arctic Sweden samples revealing taxonomic distance between samples and showing the main taxa causing the separations. Samples cluster according to habitats in all locations. Bar charts show average species composition within one habitat and

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

location with the numbers in brackets at the bottom of each chart representing sample numbers per habitat.

Figure 4: CCA analysis showing a positive correlation for the relative abundance of the two uncultured *Chlamydomonadaceae* (1) and (2) with PUFAs, *Chloromonas cf. alpina* with MUFAs and a negative correlation for *Raphidonema sempervirens* with PUFAs.

Figure 5: CCA analysis showing a positive correlation between the two uncultured *Chlamydomonadaceae* (1) and (2) and negative correlation between the uncultured *Chlamydomonadaceae* (3) and *Chloromonas cf. alpina* with secondary carotenoids. *Raphidonema sempervirens* was also positively correlated with primary carotenoids.

Figure 6: Correlations between C/N, C/P and N/P ratios and the relative abundance of analysed metabolites in the samples representing the various habitats at both locations. On average all metabolites correlated positively with the particulate C/N/P ratios. C/N ratios were correlated with higher lipid, secondary carotenoid and PUFA contents; while C/P and N/P ratios were correlated with higher lipid, secondary carotenoid and protein contents. Pearson correlations (r) and p-values (<0.05 is significant) are shown with in bold those that are highly significant. Vertical black lines in each plot indicate the respective Redfield ratio value.

Accepted Article

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

Supplementary information

Table S1: Overview of sample numbers, locations, coordinates and field measurements

Table S2: Distribution of 97% clustered OTUs aligned and assigned to *Archaeplastida* (green algae) taxa separated by habitat and locations. Values are the relative abundance of the taxa in percentage of total sequences and table shows taxa with OTUs of a minimum total observation count of 0.1%. It is important to note that values are rounded to one digit; therefore the abundance of a taxon with a value of 0 in one sample can range between 0 and 0.04%.

Table S3: Distribution of 97% clustered OTUs aligned and assigned to eukaryote taxa separated by habitat and locations. Values are the relative abundance of the taxa in percentage of total sequences. It is important to note that values are rounded to one digit; therefore the abundance of a taxon with a value of 0 in one sample can range between 0 and 0.04%.

Table S4: Distribution of 97% clustered OTUs aligned and assigned to known bacterial taxa separated by habitat and locations. Values are the relative abundance of the taxa in percentage of total sequences and figure shows taxa with >0.01% abundance. It is important to note that values are rounded to one digit; therefore the abundance of a taxon with a value of 0.0 in one sample can range between 0 and 0.04%.

Table S5: Distribution of 97% clustered OTUs aligned and assigned to archaea separated by habitat and locations. It is important to note that values are rounded to

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Accepted Article

one digit; therefore the abundance of a taxon with a value of 0 in one sample can range between 0 and 0.04%.

Table S6: Number of sequences before and after quality control, assigned to taxa and with respective diversity indices (Shannon, Simpson).

Table S7: Dissolved organic carbon (DOC), nutrients and other inorganic aqueous chemical data for Svalbard (SVA-) and Arctic Sweden (TAR-) samples separated into habitats.

Table S8: Total carbon (TC), total nitrogen (TN), total phosphorus (TP) and total sulphur (TS) values reported as % of dry weight of sample with the corresponding C/N, C/P and N/P ratios as well as $\delta^{15}N$ and $\delta^{13}C$ isotopes; samples are separated by habitat and location and the averages for each habitat and location are also shown. Samples that did not contain enough particulate material for analyses are shown as n.s. (no sample). For the Sweden (TAR) samples pollen counts are also included since they have likely contributed to higher TC and therefore C/N and C/P values.

Table S9: Bulk functional group distribution from the FTIR analyses of samples that contained enough particulate material, again separated by habitat and location and reported with averages. Main functional groups representing the lipids (CH_2 and CH_3 stretching modes between 3050 and 2800 cm^{-1}), proteins (amide I and II bands between 1700-1500 cm^{-1}) and carbohydrates (C-O-C, C-O-P, P-O-P ring vibrations between 1204-815 cm^{-1}) are reported as percentage of total functional groups.

Table S10: Dominant fatty acid compounds present in the samples separated by habitats and locations and reported as % of total fatty acid content with **B**

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

designating the **B**ranched and **A** the **A**lkane compounds. Total saturated (SFA), total monounsaturated (MUFA) and total polyunsaturated (PUFA) fatty acids are also reported in grey columns.

Table S11: Pigment composition of samples that contained enough particulate material for analysis and separated by habitats and locations. Individual pigments were quantified in ug/L and grey columns show total chlorophylls, total primary carotenoids and total secondary carotenoids in % of total pigments. Chl a = chlorophyll a, Chl b = chlorophyll b, Neo = Neoxanthin, Vio = Violaxanthin, Ant = Antheraxanthin, Lut = Lutein, Zea = Zeaxanthin, β-car = β-carotene, Ast=Astaxanthin.

Table S12: Carbohydrate compound contents in habitat samples from Svalbard reported as ug of whole compound per L of filtered sample.

Table S13: Average values, standard deviations and statistical analysis of all biological and geochemical compounds (in % of total), analysed by one-way ANOVA to reveal significant differences between the habitats within one location. Results with p-values of <0.05 were considered to be significant and are in bold, results with p-values <0.01 were considered to be highly significant and are also underlined. n.s. = no sample

Table S14: Average values and standard deviations for all geochemical compounds that revealed significant (p<0.05) differences between Svalbard and Arctic Sweden regardless of the habitats.

Figure S1: Principal component analysis of fatty acids in the Svalbard and Arctic Sweden samples revealing distance between samples and showing the main fatty

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

acid compounds causing the separations. Samples cluster according to habitats in all locations.

Figure S2: CCA analysis showing the links between algal species and various relevant geochemical parameters. The two uncultured *Chlamydomonadaceae* (1) and (2) positively correlated with increasing C/N ratios, *Chloromonas polyptera* with DOC and K, *Raphidonema sempervirens* with Fe and Mn, and *Chloromonas cf. alpina* with DOC.

Figure S3: CCA analysis showing the links between bacterial classes and various relevant geochemical parameters. *Sphingobacteria* positively correlated with DOC, C/N, C/P, N/P and K, and *Synechococcophycideae* with SO_4.

Figure S4: C/N and C/P ratios for the analysed Svalbard and Arctic Sweden samples. Lines show optimal Redfield ratios (solid line: C/N, dashed line: C/P). Most samples were above the optimal Redfield ratio for C/N but only the samples from the red snow habitats from Arctic Sweden were above the optimal C/P ratio. Samples cluster according to habitats within their respective location.

F**igure S5:** Carbon and nitrogen isotopes. $\delta^{15}N$ values were predominately negative but show no significant trend ($p>0.05$) for habitats or locations. $\delta^{13}C$ values varied over a narrow range and significant trends ($p=0.004$) were only established for habitats in Svalbard (left) where on average most red snow samples showed more negative values than the grey ice and cryoconite hole samples. No significant trends were observed for samples from Sweden (right).

Figure S6: Relative abundance of functional group corresponding to lipids and proteins showing that samples cluster according to algal habitats. Lipids and proteins

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

show highest values in red snow (specifically in the Arctic Sweden samples), whereas in the Svalbard samples lipids were significantly higher but not proteins.

Accepted Article

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

Table 1: Statistical analysis of correlations between all analysed biological and geochemical compounds in all samples. Shown are Pearson correlation factors (r) and significance of correlations (p). Correlations with $p < 0.05$ were considered to be significant. DOC=dissolved organic carbon; K = potassium, Fe = iron, Mn = manganese; C = total particulate organic carbon, N = total particulate nitrogen; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

	r	p
Algae and geochemistry		
Chloromonas cf. alpina ~ DOC	0.420	0.004
Chloromonas polyptera ~ DOC	0.593	<0.001
Chloromonas polyptera ~ K	0.424	0.002
Raphidonema sempervirens ~ Fe	0.411	0.003
Raphidonema sempervirens ~ Mn	0.402	0.004
Uncultured *Chlamydomonadaceae* (1) ~ C:N	0.337	0.019
Uncultured *Chlamydomonadaceae* (2) ~ C:N	0.456	0.001
Algae and metabolites		
Chloromonas cf. alpina ~ MUFA	0.509	<0.001
Uncultured *Chlamydomonadaceae* (1) ~ PUFA	0.596	<0.001
Uncultured *Chlamydomonadaceae* (2) ~ PUFA	0.593	<0.001
Raphidonema sempervirens ~ PUFA	-0.493	<0.001
Uncultured *Chlamydomonadaceae* (1) ~ Secondary Carotenoids	0.509	<0.001
Uncultured *Chlamydomonadaceae* (2) ~ Secondary Carotenoids	0.544	<0.001
Raphidonema sempervirens ~ Secondary Carotenoids	-0.608	<0.001
Raphidonema sempervirens ~ Primary Carotenoids	0.562	<0.001
Ancylonema nordenskiöldii ~ Antheraxanthin	0.590	0.094
Ancylonema nordenskiöldii ~ Zeaxanthin	0.979	<0.001
Uncultured *Chlamydomonadaceae* (2) ~ trans-Astaxanthin	0.509	0.001
Bacteria and geochemistry		
Sphingobacteria ~ DOC	0.567	<0.001
Sphingobacteria ~ TC	0.431	0.004
Sphingobacteria ~ C:N	0.418	0.003
Sphingobacteria ~ C:P	0.669	<0.001
Sphingobacteria ~ N:P	0.535	<0.001
Sphingobacteria ~ K	0.365	<0.001
Synechococcopycideae ~ SO4	0.398	0.005
Geochemistry and metabolites		
C:N ~ Lipids	0.457	<0.001
C:P ~ Lipids	0.832	<0.001
N:P ~ Lipids	0.782	<0.001
C:N ~ Proteins	0.356	0.011
C:P ~ Proteins	0.742	<0.001
N:P ~ Proteins	0.757	<0.001
C:N ~ Secondary carotenoids	0.428	0.002
C:P ~ Secondary carotenoids	0.246	0.108
N:P ~ Secondary carotenoids	0.630	0.075
C:N ~ PUFA	0.674	<0.001
C:P ~ PUFA	0.584	<0.001
N:P ~ PUFA	0.489	0.002
TC ~ DOC	0.588	<0.001

Accepted Article

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

This article is protected by copyright. All rights reserved.

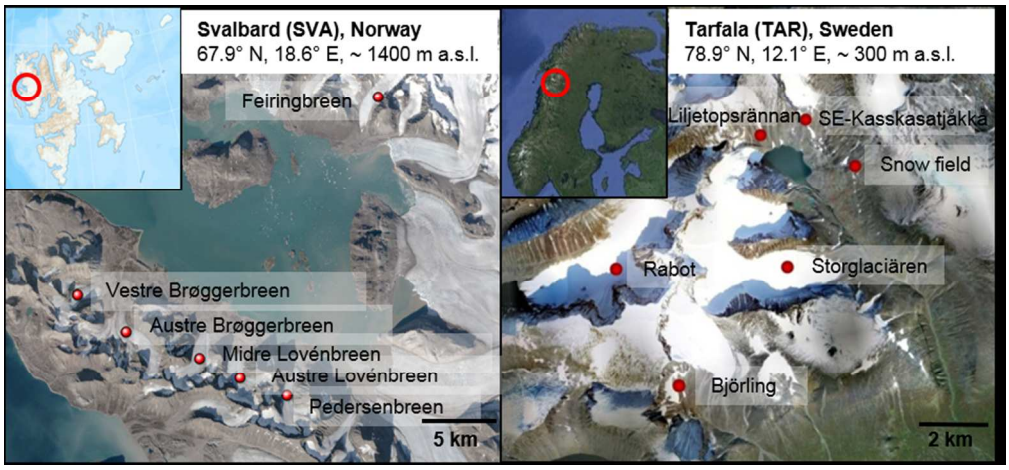

Figure 1: Map showing the 2 geographic locations and 12 glaciers and snow fields sampled in Svalbard and Arctic Sweden. At each marked site various habitats were sampled (full details see Table S1).
Figure 1
161x74mm (150 x 150 DPI)

Accepted

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

This article is protected by copyright. All rights reserved.

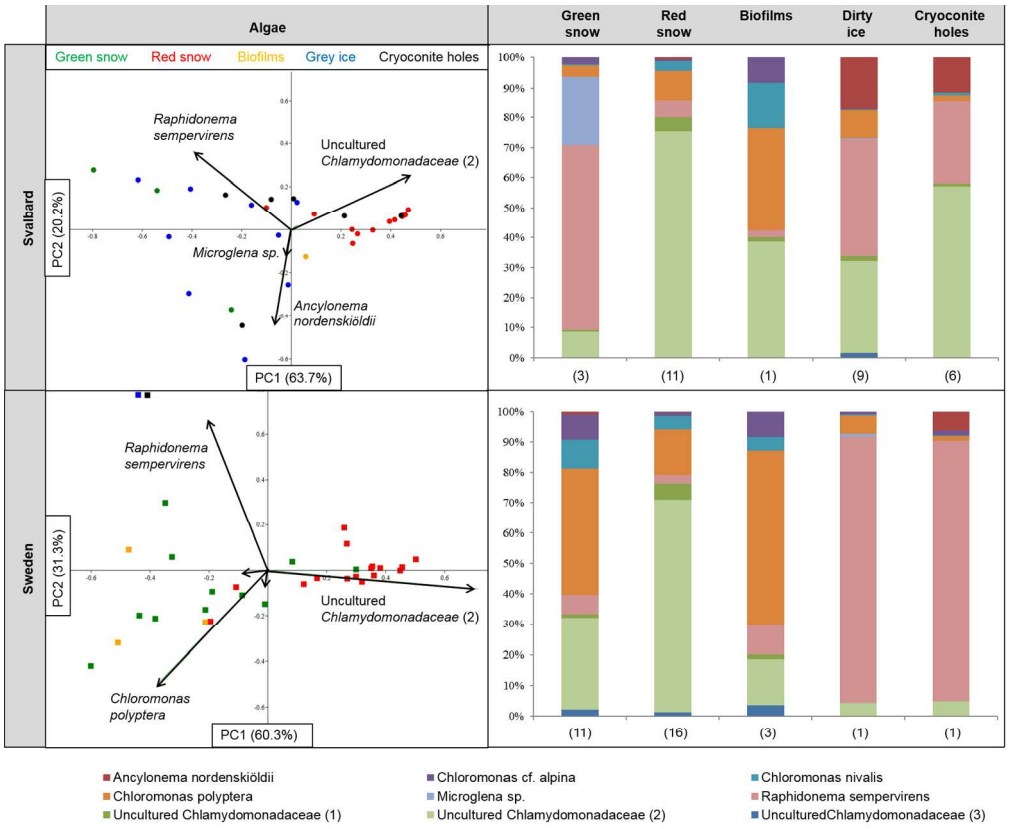

Figure 2: Principal component analysis of algal species in Svalbard and Arctic Sweden samples revealing taxonomic distance between samples and showing the main taxa causing the separations. Samples cluster according to habitats in all locations. Bar charts show average species composition within one habitat and location with the numbers in brackets at the bottom of each chart representing sample numbers per habitat.

Figure 2
491x404mm (96 x 96 DPI)

Accepted

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

This article is protected by copyright. All rights reserved.

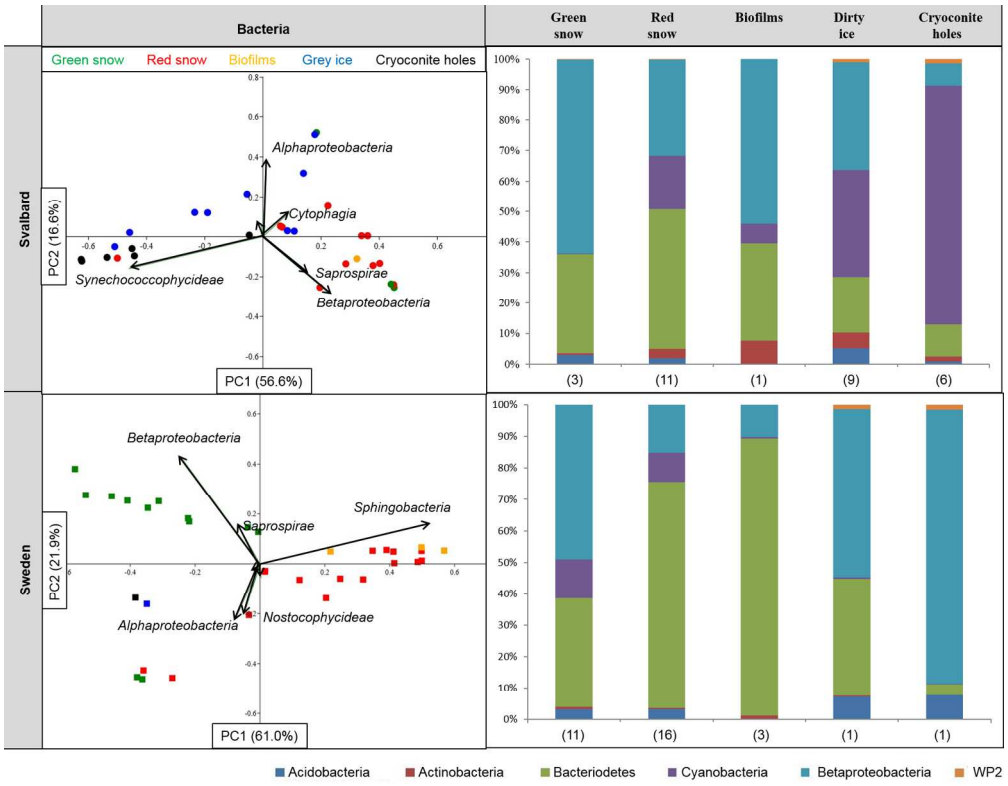

Figure 3: Principal component analysis of bacterial phyla in Svalbard and Arctic Sweden samples revealing taxonomic distance between samples and showing the main taxa causing the separations. Samples cluster according to habitats in all locations. Bar charts show average species composition within one habitat and location with the numbers in brackets at the bottom of each chart representing sample numbers per habitat.

Figure 3
489x382mm (96 x 96 DPI)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

This article is protected by copyright. All rights reserved.

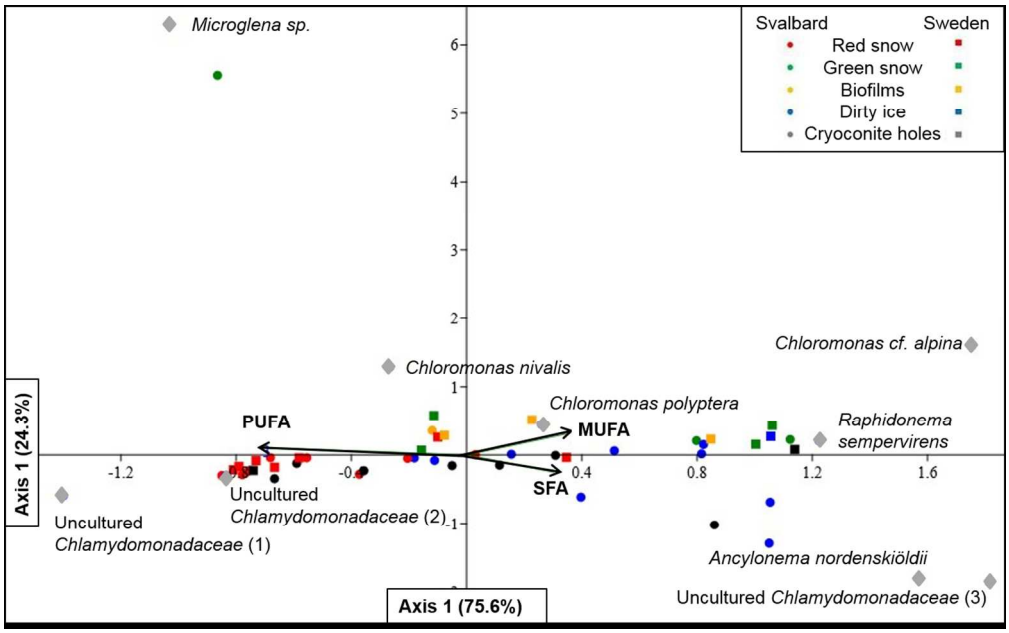

Figure 4: CCA analysis showing a positive correlation for the relative abundance of the two uncultured Chlamydomonadaceae (1) and (2) with PUFAs, Chloromonas cf. alpina with MUFAs and a negative correlation for Raphidonema sempervirens with PUFAs.
Figure 4
229x143mm (150 x 150 DPI)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

This article is protected by copyright. All rights reserved.

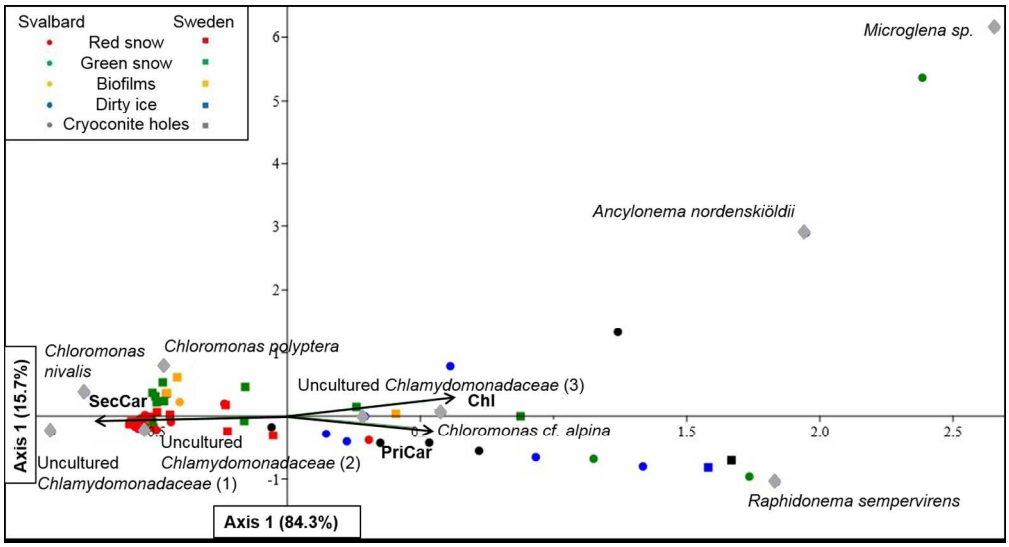

Figure 5: CCA analysis showing a positive correlation between the two uncultured Chlamydomonadaceae (1) and (2) and negative correlation between the uncultured Chlamydomonadaceae (3) and Chloromonas cf. alpina with secondary carotenoids. Raphidonema sempervirens was also positively correlated with primary carotenoids.

Figure 5
248x133mm (150 x 150 DPI)

Accepted

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

This article is protected by copyright. All rights reserved.

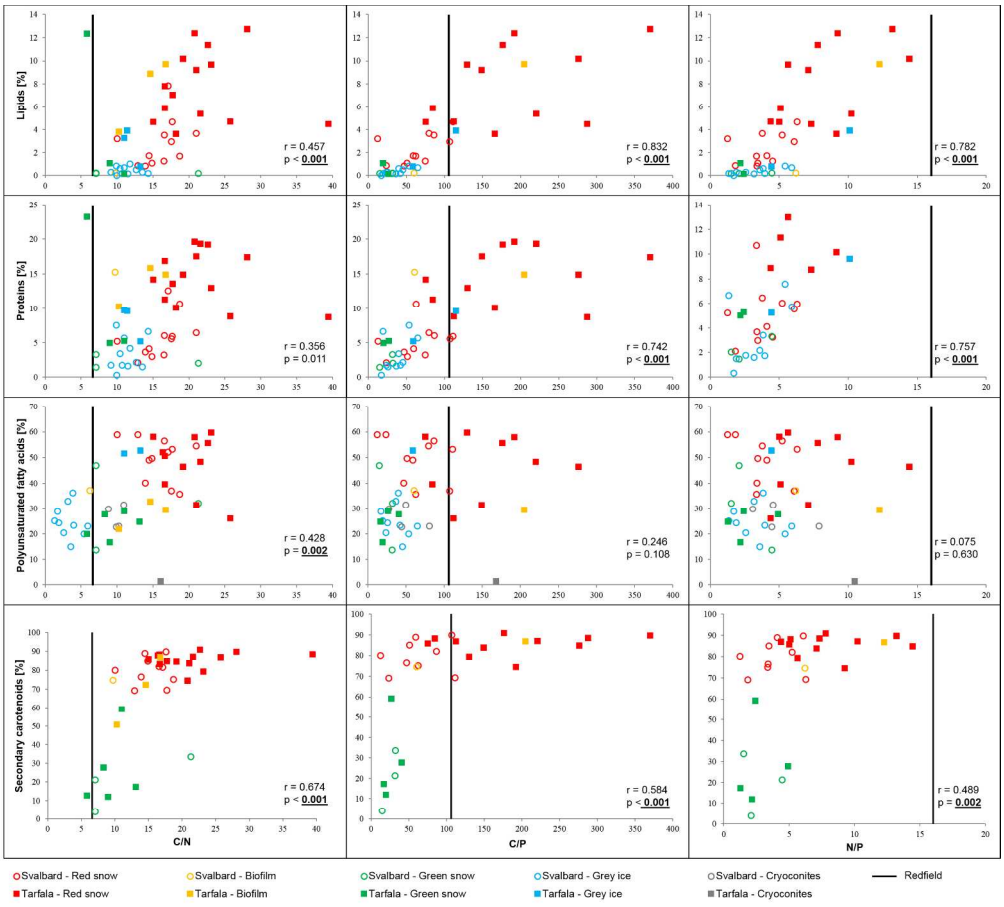

Figure 6: Correlations between C/N, C/P and N/P ratios and the relative abundance of analysed metabolites in the samples representing the various habitats at both locations. On average all metabolites correlated positively with the particulate C/N/P ratios. C/N ratios were correlated with higher lipid, secondary carotenoid and PUFA contents; while C/P and N/P ratios were correlated with higher lipid, secondary carotenoid and protein contents. Pearson correlations (r) and p-values (<0.05 is significant) are shown with in bold those that are highly significant. Vertical black lines in each plot indicate the respective Redfield ratio value.

Figure 6
640x578mm (96 x 96 DPI)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

This article is protected by copyright. All rights reserved.